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Cell Biology: Polar Expeditions for PP1

A new study shows that phospho-dependent expulsion of type-1-phosphatase (PP1) from the spindle pole by Fin1 (NIMA) kinase ensures switch-like activation of Cyclin B–Cdk1 at the G2/M transition.

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It has been known for more than twenty years that the onset of mitosis is triggered by activation of the Cyclin B–Cdk1 complex. During G2 phase of the cell cycle Cyclin B–Cdk1 is kept inactive by phosphorylation of two residues (Thr14 and Tyr15), within the ATP-binding pocket of Cdk1, by the Wee1-related kinases. At the onset of mitosis these residues are dephosphorylated by the Cdc25 phosphatase, which results in Cyclin B–Cdk1 activation. Crucially, initial activation of a small pool of Cyclin B–Cdk1 drives the activation of other Cyclin B–Cdk1 complexes by initiating a double-positive feedback loop by phosphorylating and activating Cdc25 and inactivating Wee1. Thus, Cyclin B–Cdk1 activity must reach a critical threshold before the whole pool is rapidly and globally activated, resulting in switch-like activation of Cdk1 at the onset of mitosis. New work in fission yeast, as reported in this issue of *Current Biology* by Grallert *et al.* [1], reveals that association of type 1 phosphatase (PP1) to the spindle pole, and its expulsion from the pole at the G2/M transition, play a key role in setting the threshold for this auto-amplification loop.

Several lines of evidence suggest that recruitment of mitotic regulators to the centrosome (spindle pole body in yeast) facilitates commitment of cells to mitosis. Active Cyclin B–Cdk1 complex is first detected at the centrosomes in prophase in mammalian cells [2]. Cdc25B, one of three Cdc25 isoforms in mammalian cells, is likely to catalyse initial activation of centrosomal Cyclin B1–Cdk1 as Cdc25B binds centrosomes and reducing Cdc25B protein levels by RNAi delays centrosomal Cdk1 activation and entry into mitosis [3]. In addition to Cdc25B, several centrosome-associated kinases, including Polo, Aurora A and NIMA, are also implicated in activation of Cyclin B–Cdk1 at mitotic onset. Human Polo kinase, Plk1, appears on the centrosome during G2 phase, co-incident with an increase in Plk1 activity, and inhibition of Plk1 delays, but does not block, mitotic commitment [2,3]. Activation of Plk1 in G2 phase requires phosphorylation of its kinase domain by Aurora A kinase. Notably the *Xenopus* Polo kinase, Plx1, was purified as a Cdc25 activating kinase, suggesting a role for Plx1 in amplification of Cdk1 activity [2,3]. Aurora A can also phosphorylate Cdc25B on a site that correlates with its centrosomal relocalization. However, there is no indication that Aurora A directly affects Cdc25 activity. Additionally, loss-of-function mutations in the *nimA* (never-in-mitosis A) kinase were first isolated in a screen for cell division cycle mutants in *Aspergillus nidulans* that arrest in G2 [4]. Consistently, loss of Fin1 kinase (NIMA homologue) delays Cdk1 activation in fission yeast by indirectly controlling Wee1 activity [5]. However, it remains unclear how the activities of these kinases are integrated at the spindle pole to overcome the threshold for Cdk1 auto-amplification.

The finding that the fission yeast *cut12* gene is allelic to *stf1-1* (*cut12-G71V*), a semi-dominant mutation that suppresses *cdc25-22* mutants at high temperature, provided strong genetic evidence that the spindle pole plays an important role in promoting the G2/M transition [6]. Importantly, whereas association of Plo1 kinase (Polo homologue) to Pcp1 (a centrosomin-like protein) at spindle poles normally occurs in late G2, Plo1 is recruited to spindle poles in early G2 in *cut12-G71V* mutants [6,7]. In elegant recent experiments Hagan and colleagues [8] confirm that the spindle pole promotes mitotic entry, at least in part, by stimulating the activity of Plo1 kinase. But the question is how? The same authors now reveal that glycine 71 in Cut12 is part of a bipartite docking site for protein phosphatase 1 (PP1). When association of PP1 to Cut12 is totally abolished, Plo1 kinase is prematurely recruited to the spindle pole and activated to higher levels than normal. Consequently, cells enter mitosis even in the complete absence of the Cdc25 phosphatase [1]. The authors reason that PP1 bound to Cut12 sets the threshold for Cdk1 auto-amplification in G2 by restricting spindle pole association of Plo1 kinase and by antagonising activation of Cdk1 kinase by Cdc25.

This raises the following question: how is the threshold for Cdk1 activation overcome as cells commit to mitosis? Notably, ectopic recruitment of Plo1 kinase to spindle poles in *cut12-G12V* cells depends on Fin1 kinase [2]. Hagan and colleagues provide an elegant explanation for this by showing that phosphorylation of threonine 75 and 78 in Cut12 by Cdk1 and Fin1 kinases, respectively, antagonizes binding of PP1 to Cut12 [1]. These results suggest that initial phosphorylation of Cut12 on threonine 78 by Fin1 promotes release of some PP1 that in turn results in spindle pole recruitment and activation of Plo1 kinase. This triggers local activation of Cyclin B–Cdk1 at the pole, resulting in phosphorylation of Cut12 on threonine 75, further PP1 release and so on (Figure 1). However, it is not clear if Plo1 kinase is directly involved in activating Cdc25 in fission yeast. One possibility is that the pool of PP1 that is released from Cut12 may inactivate Wee1 kinase, for example by reversing inhibitory phosphorylations on the Cdr1 and Cdr2 kinases imposed by the Pom1 kinase (Figure 1). This is not unreasonable as the Wee1, Cdr1 and Cdr2 kinase localize to numerous membrane-associated equatorial foci that surround the middle of the cell and are thus located close to the spindle pole body [9,10]. However, Hagan and colleagues show that loss of PP1 does not cause *cut12-PBSA cdc25-22* mutants to arrest at high temperature [1]. An alternative possibility is that Plo1 kinase down-regulates Wee1 activity via the Cell Geometry Network (CGN), involving the Cdr1, Cdr2 and Pom1 kinases, possibly by further local activation of Fin1 kinase at the spindle pole [5,9,10]. Clearly, further experiments will be needed to elucidate the complete circuitry of this amplification loop.

It should be noted that although Cdc25B and Plk1 are dispensable for

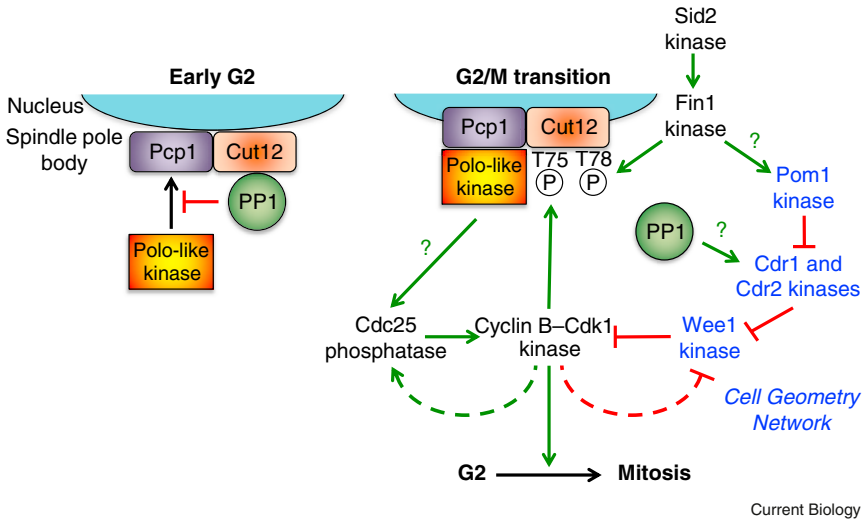

Figure 1. Priming mitotic commitment at the pole.

In early G2, PP1 is bound to Cut12 at the spindle pole and inhibits the localisation and activity of Polo-like kinase. During late G2, Sid2 kinase activates Fin1 kinase to phosphorylate Cut12, leading to expulsion of PP1 from the pole, allowing Polo-like kinase to bind and become activated. This triggers auto-amplification of Cyclin B–Cdk1 activity by activating Cdc25 phosphatase and simultaneously inactivating Wee1 kinase via the Cell Geometry Network (containing Pom1, Cdr1, Cdr2 and Wee1 kinases in blue). See text for more details. Green and red lines represent activation and inhibition, respectively, dashed lines indicate auto-amplification loops and question marks highlight poorly understood mechanisms or hypotheses.

mitotic initiation during a normal cell cycle in human cells, they are both required to initiate mitosis following recovery from cell cycle arrest induced by DNA damage [3]. Similarly, Ark1 kinase (Aurora homologue) is required to promote mitotic onset following TOR-mediated cell cycle arrest by driving recruitment of Plo1 kinase to the spindle pole [11]. It is intriguing, in this regard, that the PP1-binding site in Cut12 also contains consensus phosphorylation sites for Aurora kinase [1]. However, current evidence suggests Ark1 is not strictly required for initiation of mitosis in rapidly dividing fission yeast cells. Moreover, centrosomes are dispensable for entry into mitosis in worms and flies [12,13]. This suggests the threshold for Cdk1 auto-amplification may be greater in cells recovering from a cell cycle arrest than in cycling cells, and/or that the spindle pole is required to facilitate Cdk1 activation by raising the local concentration of Cyclin B–Cdk1 and its activators only when cells resume cell proliferation.

Regardless, it is becoming increasingly apparent that rapid local and global changes in phosphatase activity play a key role in determining the sharpness and irreversibility of cell cycle transitions [14]. For example, when tension is applied across the sister kinetochore during mitosis, the outer kinetochore is pulled away from the inner centromere, thereby separating Aurora B kinase from its substrates [15]. This is reinforced by the tension-dependent recruitment of the type 1 phosphatase (PP1) with the amino-terminus of KNL1/Spc7 [16]. This stabilizes microtubule–kinetochore interactions and silences the spindle checkpoint to promote the onset of anaphase [16,17]. Likewise, global inactivation of PP2A at the G2/M transition is as important for entry into mitosis as activation of Cyclin B–Cdk1 kinase. In this case, initial activation of Cyclin B–Cdk1, possibly at the pole, activates Greatwall kinase, which in turn phosphorylates ARPP-19/ENSA to create an inhibitor that binds and inactivates PP2A [18,19]. Thus, inactivation of PP2A and expulsion of PP1 from the spindle pole represent two faces of the same coin. In the future it will be important to determine whether this latter mechanism is conserved in mammals. Although Cut12 does not have clear homologues outside of fungi, some centrosomal proteins, such as Cep192 and Sfi1, have been identified as PP1-interacting proteins and so may act in a similar manner [20]. More generally, it will also be important to understand how PP1 and PP2A activities are interconnected, as these phosphatases are essential for maintaining normal cell division and preventing inappropriate cell proliferation, a central hallmark of cancer cells.

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Cell Division: Kinetochores SKAdaddle

Accurate chromosome segregation during cell division requires that kinetochores couple microtubule dynamics to chromosome movement. New research reveals that the kinetochore-associated Ska1 complex hangs on to depolymerizing microtubules and brings some important friends along for the ride.

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The kinetochore is a structure composed of hundreds of proteins that assembles on the centromeres of each sister chromatid during cell division. A primary function of the kinetochore is to attach chromosomes to spindle microtubules in a manner that allows force produced by microtubule dynamics to be harnessed in order to move the chromosomes. At no time during cell division is this more evident than during anaphase when kinetochores remain associated with depolymerizing kinetochore-microtubules (kt-MT). There has been an ongoing search, especially outside of yeast, for kinetochore components that are capable of coupling microtubule dynamics to chromosome movement. A recent study by Schmidt *et al.* [1] demonstrates that the Ska1 complex utilizes a conserved microtubule-binding domain (MTBD) to track depolymerizing microtubule ends and confers this activity to a core kt-MT attachment factor called the Ndc80 complex.

In yeast, the Dam1 (also known as DASH or DDD) complex is required for stable kt-MT attachments. The ten-protein Dam1 complex can associate with microtubules either as a ring around the microtubule [2,3] or as non-ring oligomers [4]. Dam1 complex tracks depolymerizing microtubules *in vivo* and *in vitro* [5,6]. Additionally, the Dam1 complex is capable of imparting tracking activity to the Ndc80 complex [7,8]. While the Ndc80 complex is highly conserved, definitive Dam1 complex homologues have never been found beyond fungal species. Consequently, there has been a mystery surrounding the mechanisms by which metazoan kinetochores efficiently couple the force generated by microtubule dynamics into chromosome movement.

The Ndc80 complex itself has been a leading candidate for the coupling factor. It has been difficult to directly assess the coupling activity of Ndc80 in cells because kt-MT attachments cannot form in its absence. *In vitro* reconstitution experiments have been more informative as Ndc80 complexes bound to beads were found to track depolymerizing microtubule ends [9,10]. Coupler found and case closed? Not quite. The fact that bead-bound Ndc80 complex only acquired tracking activity when oligomeric assemblies formed on the beads [9] raised the possibility that other coupling molecules existed in cells.

A new candidate began to emerge when Hanisch *et al.* [11] discovered that two novel proteins called Ska1 (spindle and kinetochore associated 1) and Ska2 were required for timely anaphase progression. A third member of the complex named Ska3, formerly known as C13orf3 and RAMA1 [12–15], was later discovered. In some respects, the three protein Ska1 complex behaves differently than Ndc80 as its localization to kinetochores is microtubule and Ndc80-dependent. Yet like the Ndc80 complex, the Ska1 complex is required for kt-MT attachment stability and it binds directly to microtubules *in vitro* where it can also couple bead movement to depolymerizing microtubules [16]. However, the molecular basis of these activities was uncertain because, while the carboxyl termini of Ska1 and Ska3 were found to be necessary for the complex to bind microtubules [17], the actual MTBD of the Ska1 complex had not yet been defined.

Schmidt *et al.* [1] set out to determine how the Ska1 complex interacts with microtubules. Through homology searches, the authors found the first non-vertebrate homologue of Ska1 in *Caenorhabditis elegans* (CeSka1), which exhibited the highest homology to human Ska1 (hSka1) in its carboxyl terminus. Like its human counterpart, the CeSka1 complex bound directly to microtubules and the conserved carboxyl terminus was necessary and sufficient for Ska1 to associate with microtubules *in vitro* and in cells. While depletion of the Ska1 complex caused severe chromosome congression defects, cells expressing Ska1 complex lacking the MTBD (ΔMTBD) were able to achieve metaphase alignment, although kt-MT attachments were not as stable. These findings indicate that the Ska1 complex contributes to chromosome alignment independent of its microtubule-binding activity. Interestingly, congressed chromosomes in Ska1 ΔMTBD expressing cells did not exhibit normal chromosome oscillations, suggesting that the complex is required for